UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

NOV 14 1989

MEMORANDUM

SUBJECT: Elanco Response to the Trifluralin Reregistration Standard: Animal Metabolism Studies. (MRID #'s 41233100, 41233101 and 41233102, DEB # 5927.)

FROM: Richard D. Schmitt, Ph.D., Chief
Dietary Exposure Branchh
Health Effects Division (H7509C)

Edward Zager, for

TO: Reto Engler, Ph.D., Chief
Science Analysis and Coordination Branch
Health Effects Division (H7509C)

and

L. Rossi, Chief
Reregistration Branch (H7508C)
Special Review and Reregistration Division (H7508C)

Attached are reviews of animal metabolism studies submitted by Elanco in response to the trifluralin reregistration standard. These studies were reviewed by Dynamac Corporation under supervision of Dietary Exposure Branch, HED.

The due date for this review is December 19, 1989.

These studies have undergone secondary review in Dietary Exposure Branch and have been revised to reflect the Branch policies.

Based on the results of the animal metabolism studies and in light of the Branch memorandum entitled "Guidance on When and How to Conduct Livestock Metabolism Studies." (Richard D. Schmitt to Dietary Exposure Branch Staff, 7/25/89.) the Branch considers the nature of the residue of trifluralin in animals to be adequately delineated, also, we have no scientific objectios to Elanco's request for waivers for animal feeding studies, tolerance proposals

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for meat, milk, poultry and eggs and analytical enforcement methodology for residues of trifluralin in animal products. The granting of such waivers however is the prerogative of SRRD.

If you need additional input please advise.

Attachment 1 : Review of Trifluralin Animal Metabolism Studies.

cc: With Attachment 1: R. B. Perfetti, R. Coberly (TOX), J. Burrell (FOD), Trifluralin Reregistration standard file, Trifluralin Subject File.

cc: Without Attachment: P. Fenner-Crisp (HED), M. Hawkins (HED), F. Bishop (RD), Circulation (7) and RF.

DYNAMAC
CORPORATION

Final Report

TRIFLURALIN
Task 4: DEB No. 5927 - Registrant's Response to Residue Chemistry Data Requirements

November 10, 1989

Contract No. 68-D8-0080

Submitted to:
Environmental Protection Agency
Arlington, VA 22202

Submitted by:
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TRIFLURALIN

REGISTRANT'S RESPONSE TO RESIDUE CHEMISTRY DATA REQUIREMENTS

Task - 4

BACKGROUND

The Trifluralin Guidance Document dated April, 1987 concludes that the metabolism of trifluralin in animals is not adequately understood and requires additional data depicting the distribution and nature of residues of ring-labeled [^{14}C]trifluralin in meat, milk, poultry tissues, and eggs. In response to these data requirements, Elanco Products Company has submitted two volumes of data (DEB No. 5927, 1989; MRIDs 41233101 and 41233102) which are reviewed here for their adequacy in fulfilling the outstanding data requirements.

Deficiencies Remaining to be Resolved

The remaining residue chemistry data gaps on storage stability; residue analytical methods; residue data for corn grain, sorghum grain, corn forage and fodder, alfalfa hay, flax straw, and peppermint and spearmint oil; tolerance proposals and supporting residue data for corn silage, sorghum hay and silage, alfalfa forage, cotton forage, peanut vines and hay, sugarcane forage, and sunflower forage; and processing data for potatoes, sugar beets, soybeans, citrus fruits, wheat grain, corn grain, alfalfa, cottonseed, peanuts, peppermint or spearmint hay, sugarcane, and sunflower seed that were identified in the Registration Standard have not been addressed in this submission and are still unresolved.

CONCLUSIONS PERTAINING TO THIS REVIEW

With regard to ruminant metabolism, the registrant has demonstrated that total radioactive residues (TRR) are <0.01 ppm in muscle, fat, kidney, and milk, and 0.014 ppm in liver from cattle that were administered [^{14}C]trifluralin at a level equivalent to 10x the maximum theoretical dietary levels of 0.875 ppm for beef cattle and 1.7 ppm for dairy cows (refer to the Trifluralin Residue Chemistry Chapter dated 7/12/85 for details of theoretical dietary intake calculations). It is apparent that radioactive residues resulting from dosing at 1x would be considerably lower than <0.01 ppm in all edible tissues and milk. Radioactive residues in tissues and milk from the animals dosed at 100x were partitioned between nonpolar and polar solvents and aqueous and unextractable residues were subjected to hydrolysis procedures. Limited characterization of residues in fat, milk, and liver was accomplished. The metabolite TR-14 was identified in liver and accounted for ca. 8% of the TRR. In fat, ca. 8% of the TRR was identified as TR-4 and in milk, trifluralin, TR-2, and TR-7 each accounted for 2-3%. The following metabolites were thought to be present in tissues and milk based on the elution of radioactivity from silica gel column chromatography of tissue fractions compared with that of fractions from urine in which metabolites were identified using thin-layer chromatography: (i) TR-6 and TR-14 in milk; (ii) in liver, TR-5, TR-6, TR-7, and desethyl TR-14; (iii) TR-42 and TR-44 in kidney; and (iv) in fat, trifluralin, TR-6, and TR-14.

Radioactive residues were <0.003 ppm (nondetectable) each in poultry muscle and skin plus fat, 0.004 ppm in liver, and <0.001 ppm (nondetectable) in eggs from hens administered [^{14}C]trifluralin at a level in the diet equivalent to 1x the maximum theoretical dietary intake of 0.05 ppm. Residues in tissue and egg samples from hens dosed at 1,000x were fractionated and the solids hydrolyzed, but attempts to identify metabolites using TLC or HPLC were unsuccessful. The registrant postulated, based on the polarities of residues separated on silica gel column chromatography, that unchanged trifluralin could be present in muscle; TR-2, TR-4, and TR-19 could be present in liver; skin/fat could contain trifluralin and TR-2; and that TR-3, TR-5, TR-6 TR-7, and TR-14 might be present in eggs.

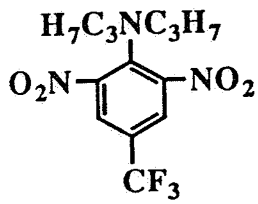
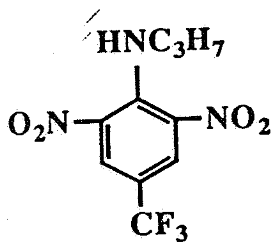
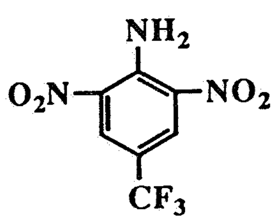
The qualitative nature of the residues in animals is adequately understood. More thorough characterization of radioactive residues in bovine and poultry tissues, milk, and eggs is not required given the low levels in these samples expected to result from the maximum theoretical dietary intake. We note that the weights of the hens and the dairy cow and the amount of feed consumed per day by the steers and the hens were not reported. This information is needed to validate the feeding levels reported in the submitted data.

The identified and putative metabolites of trifluralin in animals and plants are illustrated in Table 1.

RECOMMENDATIONS

The registrant should report the weights of the hens and the dairy cow and the amount of feed consumed per day by the steers and the hens; the feeding levels may need to be validated should the theoretical dietary intake of trifluralin residues fluctuate in the future.

Table 1. Trifluralin and its known and putative metabolites in plants[a] and animals.

Code	Chemical name Structure	Substrate	MRID[b] **Common name**
I	α,α,α-Trifluoro-2,6-dinitro-N,N-dipropyl-p-toluidine		
		Carrot root	00093553
		Carrot top	00093553
		Corn forage	41179001
		Mustard leaf	41179002
		Mustard root	41179002
		Peanut plant	None
		Sweet potato	None
		Hen muscle, skin/fat, and eggs (putative)	41233101
		Beef fat (putative)	41233102
		Cow's milk	41233102
			Trifluralin (TR-1)
II	α,α,α-Trifluoro-2,6-dinitro-N-propyl-p-toluidine		
		Carrot root	00093553
		Carrot top	00093553
		Corn forage	41179001
		Mustard root	41179001
		Peanut plant	None
		Cow's milk	41233102
		Hen liver and skin/fat (putative)	41233101
			TR-2
III	α,α,α-Trifluoro-2,6-dinitro-p-toluidine		
		Corn forage	41179001
		Mustard root	41179002
		Eggs (putative)	41233101
			TR-3

(Continued)

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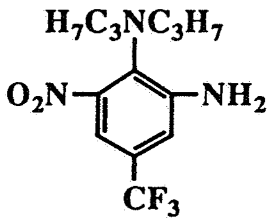
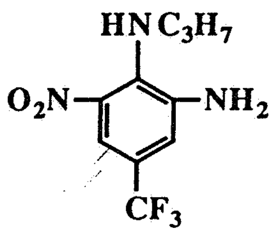
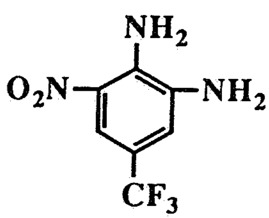
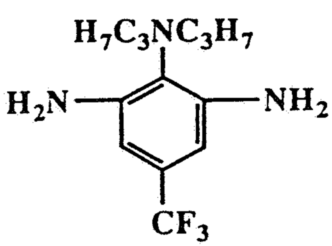
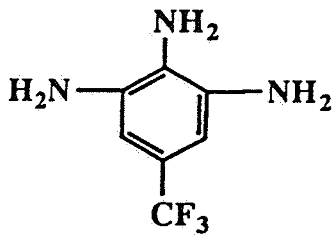
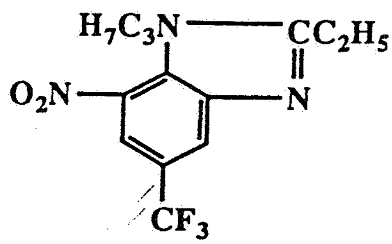
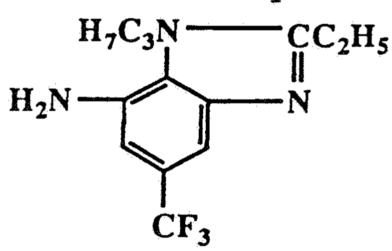
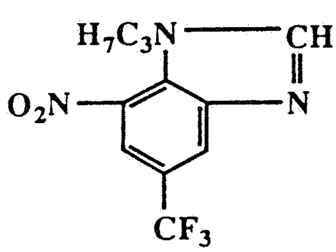
Table 1. Trifluralin and its metabolites (continued).

Code	Chemical name Structure	Substrate	MRID[b] Common name
IV	α,α,α-Trifluoro-5-nitro-N^4,N^4-dipropyltoluene-3,4-diamine 	Corn forage	41179001
		Peanut plant	None
		Beef fat	41233102
		Hen liver (putative)	41233101
			TR-4
V	α,α,α-Trifluoro-5-nitro-N^4-propyltoluene-3,4-diamine 	Carrot root	00093553
		Corn forage	41179001
		Mustard root	41179001
		Beef liver (putative)	41233102
		Eggs (putative)	41233101
			TR-5
VI	α,α,α-Trifluoro-5-nitrotoluene-3,4-diamine 	Corn forage	41179001
		Peanut plant	None
		Sweet potato	None
		Beef liver, fat; cow's milk (putative)	41233102
		Eggs (putative)	41233101
			TR-6
VII	α,α,α-Trifluoro-N^4,N^4-dipropyltoluene-3,4,5-triamine 	Mustard root	41179001
		Cow's milk	41233102
		Beef liver (putative)	41233102
		Eggs (putative)	41233101
			TR-7

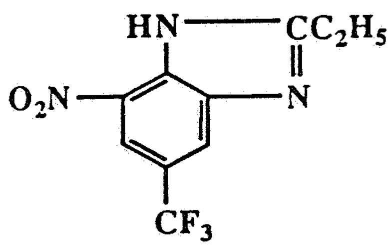
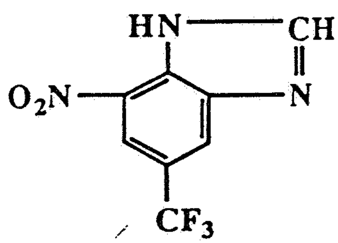
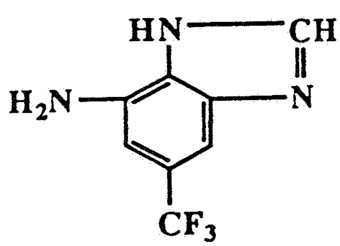
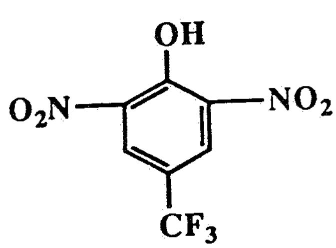
(Continued)

Table 1. Trifluralin and its metabolites (continued).

Code	Chemical name Structure	Substrate	MRID[b] **Common name**
VIII	α,α,α-Trifluoro-toluene-3,4,5-triamine 	Cattle feces Mustard root	None 41179002 **TR-9**
IX	2-Ethyl-7-nitro-1-propyl-5-(trifluoro-methyl)-benzimidazole 	(putative)	**TR-13**
X	7-Amino-2-ethyl-1-propyl-5-(trifluoromethyl)benzimidazole 	Mustard root Beef liver Beef fat (putative) Cow's milk (putative) Eggs (putative)	41179001 41233102 41233102 41233102 41233101 **TR-14**
XI	7-Nitro-1-propyl-5-(trifluoromethyl)benzimidazole 	Mustard root Beef liver, kidney (putative)	41179002 41233102 **desethyl TR-14; Metabolite D; TR-17**

(Continued)

Table 1. Trifluralin and its metabolites (continued).

Code	Chemical name / Structure	Substrate	MRID[b] / Common name
XII	2-Ethyl-7-nitro-5-(trifluoromethyl)benzimidazole 	Corn forage	41179001 / **TR-15**
XIII	7-Nitro-5-(trifluoromethyl)-benzimidazole 	Corn forage Corn forage	41179001 41179001 / **TR-18**
XIV	7-Amino-5-(trifluoromethyl) benzimidazole 	Poultry liver (putative)	41233101 / **TR-19**
XV	α,α,α-Trifluoro-2,6-dinitro-p-cresol 	Corn forage	41179001 / **TR-20**

(Continued)

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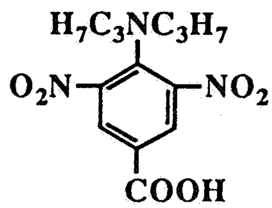
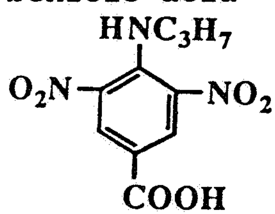
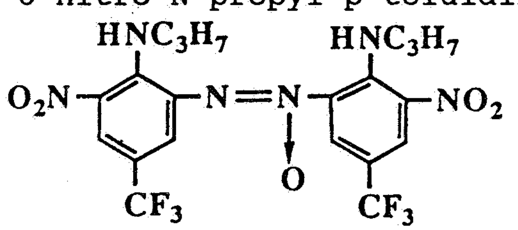
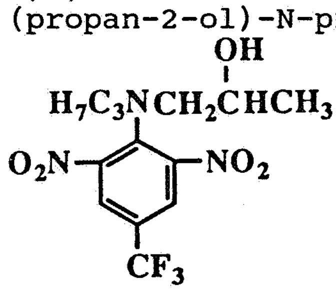
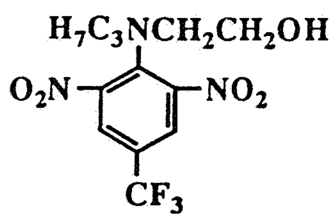
Table 1. Trifluralin and its metabolites (continued).

Code	Chemical name Structure	Substrate	MRID[b] **Common name**
XVI	4-(Dipropylamino)-3,5-dinitrobenzoic acid 	Carrot root Corn forage	00093553 41179001 **TR-21**
XVII	3,5-Dinitro-4-(propylamino)-benzoic acid 	Mustard leaf Mustard root	41179002 41179002 **TR-22**
XVIII	2,2'-Azoxybis(α,α,α-trifluoro-6-nitro-N-propyl-p-toluidine) 	Mustard root	41179002 **TR-28**
XIX	α,α,α-Trifluoro-2,6-dinitro-N-(propan-2-ol)-N-propyl-p-toluidine 	Corn forage Mustard root	41179001 41179002 **TR-41**
XX	α,α,α-Trifluoro-2,6-dinitro-N-(propan-3-ol)-N-propyl-p-toluidine 	Mustard root Beef kidney (putative)	41179002 41233102 **TR-42**

(Continued)

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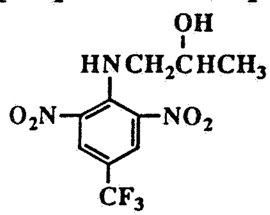
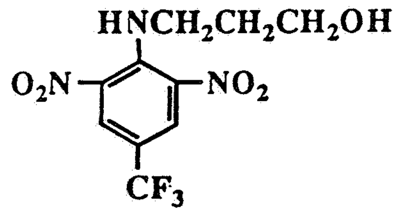
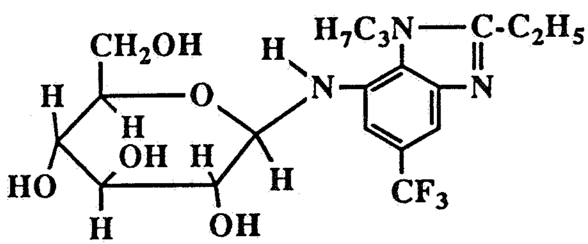
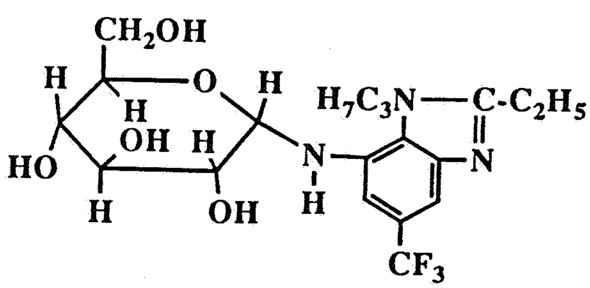
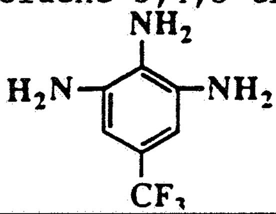
Table 1. Trifluralin and its metabolites (continued).

Code	Chemical name Structure	Substrate	MRID[b] **Common name**
XXI	α,α,α-Trifluoro-2,6-dinitro-N-(propan-2-ol)-p-toluidine 	Corn forage Mustard root	41179001 41179002 **TR-43**
XXII	α,α,α-Trifluoro-2,6-dinitro-N-(propan-3-ol)-p-toluidine 	Beef kidney (putative)	41233102 **TR-44**
XXIII	N-[2-Ethyl-1-propyl-5-(trifluoromethyl)-1H-benzimidazol-7-yl]-ß-D-gluco-pyranosylamine 	Corn fodder Corn forage Corn silage	41179001 41179001 41179001 **C-1**
XXIV	N-[2-Ethyl-1-propyl-5-(trifluoromethyl)-1H-benzimidazol-7-yl]-α-D-gluco-pyranosylamine 	Corn fodder Corn forage Corn silage	41179001 41179001 41179001 **C-2**

(Continued)

Table 1. Trifluralin and its metabolites (continued).

Code	Chemical name Structure	Substrate	MRID[b] **Common name**
XXV	α,α,α-Trifluoro-N^4,N^4-di-(n-propyl)-toluene-3,4,5-triamine 	Peanut plant Sweet potato	None None

[a] This table includes findings from studies reviewed in the Trifluralin Residue Chemistry Chapter dated 7/12/85 in addition to the studies discussed in this review.

[b] Where no MRID number is cited, the finding of the metabolite in the substrates listed was attributed, in the Residue Chemistry Chapter, to a published study by B.K. Biswas and W. Hamilton, 1969 (Weed Sci. 17:206-221).

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DETAILED CONSIDERATIONS

Administration of [^{14}C]trifluralin to beef and dairy cattle

Elanco Products Co. (1989; MRID 41233102) submitted data pertaining to the metabolism of uniformly ring-labeled [^{14}C]trifluralin (radiochemical purity >97%) in ruminants. One Hereford and one crossbred steer were used for the tissue studies and a lactating Holstein cow was used for the milk study. Details of the dosing regimen are summarized below.

Table 2. Administration of [^{14}C]trifluralin to beef and dairy cattle.

Animal	Dietary level ppm	Dietary level f(x)	Specific activity dpm/ug	Specific activity uCi/mg	Days Dosed
Hereford steer	0.875	10x	6,660	3.0	5
Crossbred steer	8.75	100x	4,418	1.99	3
Holstein cow	1.7	10x	2,220	1.0	5
Holstein cow	17	100x	440	0.2	3

The animals received [^{14}C]trifluralin and a small amount of feed in a gelatin capsule via a surgically fitted fistula; the dose was delivered directly into the rumen. The Hereford and crossbred steers weighed 206 and 234.5 kg, respectively, and were administered 4.86 and 55.1 mg of [^{14}C]trifluralin per day; consumption of feed at 2.7% of body weight per day was assumed. A single Holstein cow was used for the milk studies; 36 hours following the end of the 10x dosing period, radioactivity in milk had decreased to the pre-dose level and dosing at 100x was initiated. The cow was administered 32.6 mg per day for the 10x dose and 326 mg for the 100x dose; the weight of the animal was not reported. Feed consumption was assumed to be 21.36 kg/day, the average consumption by the dairy animal during an 18-day period prior to dosing.

Radioactivity eliminated in urine and feces of the steers was monitored during the dosing period; no data regarding the ^{14}C-activity levels in urine and feces from the dairy cow were reported. Milk was collected twice daily and stored frozen. Steers were sacrificed ca. 12 hours after the final dose and samples of liver, lean muscle, kidney, and fat were collected and stored frozen prior to preparation and analysis.

Total radioactive residue (TRR)

Frozen samples of muscle, liver, and kidney were ground and subsamples were digested for "several days" in tissue solubilizer prior to radioanalysis by liquid scintillation spectrometry

(LSS). Fat samples were melted at ca. 80 C and analyzed by LSS. Feces were blended with water, then radioassayed. Milk and urine samples were analyzed directly by LSS. The results of tissue and milk radioanalysis are summarized in Table 3.

Table 3. Total radioactive residues (TRR) in tissue extracts and milk from cattle dosed with [^{14}C]trifluralin at 10 and 100x the theoretical maximum dietary intake level.

Tissue	ppm trifluralin equivalents 10x	100x
Liver	0.014	0.145
Fat	0.004	0.015
Kidney	0.004	0.048
Muscle	<0.0006	0.003
Milk	0.0016	0.0111

Residues in milk were 0.0015-0.023 ppm (mean, 0.0016 ppm) in samples collected days 1 through 4 during the 10x dosing and 0.009-0.131 ppm (mean, 0.0111 ppm) in samples collected during the 100x dosing period (days 1 through 3); the mean figures are listed in Table 3.

Extraction

Liver: Residues in liver (samples from animals dosed at 100x) were extracted by refluxing a finely ground subsample in methanol/water (70:30); the solids were removed by filtration, radioassayed, and subjected to acid hydrolysis as described below. Methanol was removed from the methanol water extract and the residues partitioned to ethyl acetate at pH 8. The aqueous fraction was then adjusted to pH 2 and extracted again with ethyl acetate. The ethyl acetate extracts were analyzed using silica gel chromatography. The remaining aqueous phase was subjected to enzyme hydrolysis followed by further extraction and separation of liberated bound residues. Of the TRR in liver, 60% was extracted initially into methanol:water and 40% was unextracted.

The extracted aqueous fraction was incubated at pH 5 with a glucuronidase-aryl sulfatase mixture at 37 C overnight, then the residues partitioned to ethyl acetate at pH 8 and 2. The aqueous fraction contained 16.7% of the TRR, ca. 5% of which was rendered ethyl acetate soluble by enzyme hydrolysis. The remaining aqueous fraction from enzyme hydrolysis was refluxed in 3 N hydrochloric acid and partitioned with ethyl acetate at pH 8 and 2.

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Unextracted solids from the initial methanol/water extraction were refluxed in 3 N hydrochloric acid, solids removed and radioassayed, and the hydrolyzed residues were extracted into ethyl acetate at pH 1 and pH 8. The acid hydrolysis liberated ca. 27% of the TRR from the solids. The pH 8 ethyl acetate extract was pooled with those from the enzyme-hydrolyzed fraction; likewise, the acidic ethyl acetate extracts were pooled. Basic and acidic ethyl acetate residues were analyzed using silica gel column chromatography. The aqueous fraction from the acid-hydrolyzed solids was analyzed on a XAD column.

Table 4. Radioactivity in fractions of liver from cattle dosed with [^{14}C]trifluralin at 100x.

Fraction	Distribution of Radioactivity % of TRR	ppm
From initial methanol/water reflux:		
ethyl acetate pH 2	5.3	0.0077
ethyl acetate pH 8	34.7	0.0503
Pooled from hydrolyzed aqueous and solid fractions:		
ethyl acetate pH 2	5.6	0.0081
ethyl acetate pH 8	10.0	0.0145
Aqueous from enzyme hydrolysis:	10.4	0.0151
Aqueous after acid hydrolysis of solids:	16.3	0.0236
Total extracted	82.3	0.1193
Unextracted hydrolyzed solids:	11.9	0.0173
Total	94.2	0.1366

Kidney: Residues in kidney from the animal dosed at 100x were extracted with methanol/water and partitioned to ethyl acetate at pH 2 and 8 as described for liver residues. Likewise as previously described, the remaining aqueous phase was subjected to enzyme hydrolysis followed by acidic and neutral ethyl acetate partitioning. Of the 25% of the TRR in the initial extracted aqueous fraction, 11% of the TRR was made soluble in ethyl acetate by enzyme hydrolysis. Additional results are summarized in Table 5.

Table 5. Radioactivity in fractions of kidney from cattle dosed with [^{14}C]trifluralin at 100x.

Fraction	Distribution of Radioactivity % of TRR	ppm
initial methanol/water extract:		
ethyl acetate pH 2	24.3	0.0117
ethyl acetate pH 8	20.8	0.0099
hydrolyzed aqueous:		
ethyl acetate pH 2	2.7	0.0013
ethyl acetate pH 8	8.3	0.0039
extracted hydrolyzed aqueous	12.5	0.0060
Total extracted	68.6	0.0329
unextracted (solids)	29.1	0.0140
Total recovered	97.7	0.0469

Fat: Coarsely ground fat from the 100x treated steer was dissolved in hexane at 50 C. The hexane solution was filtered to remove connective tissue and partitioned to acetonitrile, and the acetonitrile fraction subjected to silica-gel column chromatography. The results of extraction of residues in fat are illustrated in Table 6.

Table 6. Radioactivity in fractions of fat from cattle dosed with [^{14}C]trifluralin at 100x.

Fraction	Distribution of Radioactivity % of TRR	ppm
hexane	19.3	0.0029
acetonitrile	68.8	0.0103
Total extracted	88.1	0.0132
unextracted (connective tissue)	11.2	0.0017
Total recovered	99.3	0.0149

Milk: Three samples of milk (volumes unreported) collected during the period of 100x dosing when residues were at steady

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state were pooled for extraction and analysis; the TRR of these samples was 0.0102, 0.0107, and 0.0131 ppm. The TRR in the pooled sample was not reported; the value of 0.0111 ppm given as the steady state mean is used in this review as the TRR of the pooled, extracted sample. The pooled sample of milk was stirred with acetone to precipitate protein and filtered, and the acetone was evaporated. The remaining aqueous fraction was partitioned with ethyl acetate, the ethyl acetate removed, and the residues partitioned to hexane and acetonitrile. The final acetonitrile fraction contained 57.9% of the TRR (ca. 0.0064 ppm) and 4.3% (ca. 0.0005 ppm) was in the final hexane fraction; 62.2% of the TRR was in these two fractions. Radioactivity in the extracted aqueous fraction and the acetone precipitate was not reported. The acetonitrile fraction was analyzed further using silica gel column chromatography.

<u>Urine</u>: Residues in urine were extracted with ethyl acetate at pH 2 and 8. The TRR in urine and the extraction efficiency were not reported. The neutral ethyl acetate extract was subjected to silica gel chromatography and the peaks were analyzed further by thin-layer chromatography (TLC).

<u>Characterization of Residues</u>

The behavior of ^{14}C-residues in tissues and milk on column chromatography was examined, but thorough identification of metabolites was attempted only in urine fractions. Limited TLC analyses were conducted on fractions from fat and milk. The presence of specific metabolites in tissues was judged primarily from comparison of the elution profiles of tissue fractions with those of urine fractions which were subsequently analyzed by TLC.

The structures of the following isolated metabolites from urine, fat, and milk were verified by mass spectral analysis: TR-5, TR-7, TR-14, and desethyl TR-14 (also known as Metabolite D and identical to the compound designated TR-17 in MRID 41179002 in a metabolism study on mustard plants).

<u>Column chromatography</u>

Residues applied to silica gel columns were eluted with an increasingly polar, nonlinear gradient progressing from hexane to toluene to ethyl acetate to 5% aqueous methanol, followed by a final elution with 5% glacial acetic acid in methanol. Fractions eluting in the same peak or on peaks of similar polarity were pooled prior to analysis for radioactivity using LSS.

Column chromatography of the neutral (pH 8) ethyl acetate-soluble residues from liver revealed seven components each containing 1-6.3% (0.0014-0.0091 ppm) of the TRR; total recovery was 23.9% of the TRR. The study authors contend that <1% of the TRR eluted in the fraction where trifluralin per se would be expected. In analyses of other liver fractions, four peaks accounting for 0.7-1.8% of the TRR were resolved from column chromatography of the acidic (pH 2) ethyl acetate fraction, the pooled neutral fraction from hydrolyzed residues was separated into five peaks containing 0.5-3.9% of the TRR, and the corresponding pooled acidic fraction yielded a single major peak with 1.1% of the TRR. The residues in the liver fractions were not characterized using TLC. Based on comparisons with the elution profiles of corresponding urine fractions, for which metabolites were identified on TLC, the authors contend that TR-14 was the residue in a major peak from the neutral ethyl acetate fractions of unhydrolyzed and hydrolyzed liver extracts (ca. 7.7% of the TRR, equivalent to 0.0112 ppm). The presence of TR-14 in the neutral liver extract was confirmed by HPLC, although data from this analysis were not provided. It was also suggested that, based on comparison with urine fractions, that the metabolites TR-5, TR-7, TR-6, and desethyl TR-14 might be present in liver extracts.

Column chromatography of the neutral ethyl acetate fraction from kidney samples resolved two major peaks representing 1.7 and 2.3% of the TRR; the corresponding chromatographic peaks from urine extracts contained TR-42 or TR-44 and desethyl TR-15, respectively.

The acetonitrile extract of fat resolved into seven major radioactive fractions, none containing more than 10.7% of the TRR. Comparison of these peaks with those from urine, suggested the presence of trifluralin per se, TR-6, and TR-14 in peaks containing 5.4, 5.3, and 10.7% of the TRR, respectively. Another peak from the acetonitrile fraction was analyzed using TLC.

The acetonitrile fraction of milk separated into seven radioactive peaks. Two of these fractions, containing 5.6 and 13% of the TRR, were thought to consist of TR-6 and TR-14, based on comparison with the results from urine. Three additional fractions from the acetonitrile extract were subjected to TLC.

Thin-layer chromatography (TLC) of residues

The neutral ethyl acetate-soluble residues in urine were separated into six major peaks from silica-gel chromatography. TLC of these peaks was conducted using either hexane:methanol (97:3), toluene:methanol (90:10), chloroform:methanol:glacial acetic acid (75:25:1), or butanol:water:glacial acetic acid (60:25:15). Radioactive zones were located by autoradiography and compounds identified by cochromatography with reference compounds (trifluralin, TR-2, -2, -4, -5, -6, -7, -14, -42, and -44) or by comparison with R_f values of standards. The radioactivity represented by individual TLC zones was not quantified.

^{14}C-Residues identified in urine were TR-5, TR-6, TR-7, TR-14, TR-42, and TR-44. An additional metabolite was isolated and designated Metabolite D; mass spectral analysis identified this compound as desethyl TR-14.

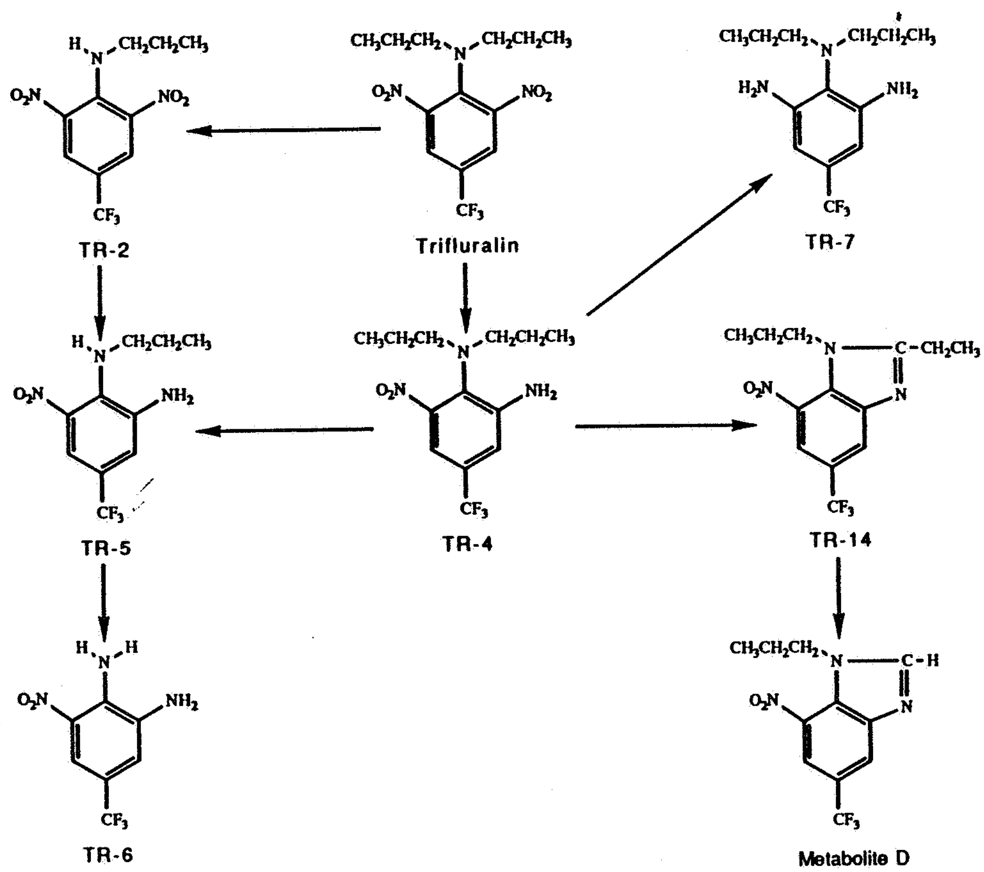
A peak from column chromatography of residues in the acetonitrile extract of fat, accounting for 8.3% of the TRR, was found via TLC to contain TR-4. Trifluralin per se was identified by TLC of one radioactive peak from the acetonitrile extract of milk, accounting for 2% of the TRR; two other peaks, containing 3.2 and 2.5% of the TRR, consisted of TR-2 and TR-7, respectively, based on TLC analysis. It should be noted that depictions of TLC plates and information regarding the specific solvents used for TLC of fat and milk fractions were not provided.

Summary of ruminant metabolism

The registrant has demonstrated that total radioactive residues (TRR) are <0.01 ppm in muscle, fat, kidney, and milk, and 0.014 ppm in liver from cattle that were administered [^{14}C]trifluralin at a level equivalent to 10x the maximum theoretical dietary levels of 0.875 ppm for beef cattle and 1.7 ppm for dairy cows. It is apparent that residues resulting from dosing at 1x would be considerably lower than <0.01 ppm in all edible tissues and milk. Radioactive residues in tissues and milk from the animals dosed at 100x were partitioned between nonpolar and polar solvents and aqueous and unextractable residues were subjected to hydrolysis procedures. Limited characterization of residues in fat, milk, and liver was accomplished. The metabolite TR-14 was identified in liver and accounted for ca. 8% of the TRR. In fat, ca. 8% of the TRR was identified as TR-4 and in milk, trifluralin, TR-2, and TR-7 each accounted for 2-3%. The following metabolites were thought to be present in tissues and milk based on the elution of radioactivity from silica gel column chromatography of tissue fractions compared with that of fractions from urine in which metabolites were identified using thin-layer chromatography: (i) TR-6 and TR-14 in milk; (ii) in liver, TR-5, TR-6, TR-7, and desethyl TR-14; (iii) TR-42 and TR-44 in kidney; and (iv) in fat, trifluralin, TR-6, and TR-14. More thorough characterization of radioactive residues in bovine tissues and milk is not required given the low levels in these samples expected to result from the maximum theoretical dietary intake. However, the registrant needs to submit additional data regarding the dosing regimen, specifically, the weight of the dairy cow and the amount of feed consumed per day by the steers.

Trifluralin and its metabolites in plants and animals are depicted in Table 1. The presence of a compound in any tissue is considered putative if based solely on comparison with results from urine analysis.

Reproduced below from the submission (MRID 41233102) is the pathway proposed by the registrant for metabolism of trifluralin in ruminants.



Administration of [^{14}C]trifluralin to laying hens

Elanco Products Company (1989; MRID 41233101) submitted data pertaining to the metabolism of trifluralin in poultry. Ring-labeled [^{14}C]trifluralin (specific activity of 8.95 uCi/mg and radiochemical purity >98%) diluted to 2 uCi/mg and 0.05 uCi/mg with unlabeled trifluralin was mixed with chicken feed (AN73CK) to achieve dietary equivalents of 0.05 ppm (1x the maximum residue level expected from feed items bearing residues), 0.5 ppm (10x), and 50 ppm (1,000x). The 1x and 10x doses were fed to Whiterock laying hens (five hens per group) for 5 days and the 1,000x ration was fed to ten hens for ten days. An additional group of hens (number not specified) were fed a control ration. Feed and water were provided *ad libitum*. The amount of feed consumed daily by hens was not reported. Eggs were collected daily from each group but not from every hen. Six hours prior to slaughter, the experimental rations were replaced with the control ration in the 1x and 10x groups. The 1,000x group was maintained on treatment until slaughter to maximize tissue residues. Liver, muscle (thigh and breast), skin (with subcutaneous fat) and eggs were analyzed separately (by bird) in the 1x and 10x groups and were pooled (by treatment) in the 1,000x group. Feces were collected, although the collection schedule was not reported.

Total Radioactive Residues

Muscle, liver, and skin samples were digested in tissue solubilizer for "several" days and egg homogenates were combined with Aquasol. Radioactivity of solubilized tissues was determined by combustion followed by liquid scintillation spectrometry (LSS). Total radioactivity in muscle, skin and liver of hens is presented in Table 7. Radioactivity was highest in liver of hens fed [^{14}C]trifluralin compared to other tissues analyzed. Residues of trifluralin were not detected in muscle and skin in the 1x treated group or in muscle from the 10x treated group. Radioactivity was at or near the detection limit in eggs from hens fed the 1x and 10x rations (Table 7). Levels of radioactivity in eggs from hens fed the 1,000x ration increased over time.

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Table 7. Total radioactivity in eggs and tissues from hens fed [^{14}C]trifluralin at levels equivalent to 0.05, 0.5 and 5 ppm in the diet (1x, 10x and 1,000x).

Tissue	ppm		
	1x	10x	1,000x
Muscle	NDR[a]	NDR	0.145
Skin/fat	NDR	0.002	0.468
Liver	0.004	0.014	2.489
Eggs			
Day			
1	NDR[a]	NDR	NDR
2	NDR	NDR	0.032
3	NDR	0.001	0.089
4	NDR	0.002	0.190
5	NDR	0.001	0.220
6	--[b]	--	0.346
7	--	--	0.409
8	--	--	0.474
9	--	--	0.463
10	--	--	0.533

[a]No detectable residues; detection limit was 0.003 ppm for tissues and 0.001 ppm for eggs.
[b]Experiment terminated on day 5.

Extraction

Pooled samples of tissues and eggs from the treatment group dosed at 1,000x were used for characterization of residues.

Residues in muscle were extracted into acetone then methanol. Methanol-soluble and acetone-soluble fractions were combined and partitioned between hexane and acetonitrile. The acetonitrile-soluble fraction was characterized by silica-gel column chromatography. The solids were then refluxed in, sequentially, methanol:1 N sodium hydroxide (3:1), methanol:water:1 N sodium hydroxide (6:1:1), and methanol:water:1 N hydrochloric acid (6:1:1:). Radioactivity in the combusted insoluble residue and extracts were determined by LSS.

Liver tissue was blended with acetone and the acetone-insoluble residue was extracted with methanol. The acetone- and methanol-soluble residues were combined and partitioned between hexane and acetonitrile. Hexane was evaporated from the hexane-soluble fraction and the resulting oil residue was diluted with methanol and combined with the acetonitrile-soluble fraction. The combined sample was characterized by column chromatography. The unextractable residues were sequentially extracted with basic and acidic methanol as described for muscle. The first methanol/sodium hydroxide fraction was neutralized with hydrochloric acid; a precipitate was removed by filtration and the filtrate analyzed by silica gel column chromatography.

Residues in skin and fat were extracted with hexane and then methanol. The hexane-soluble fraction was partitioned with acetonitrile, and the methanol- and acetonitrile-soluble fractions were combined and analyzed by column chromatography. The methanol-insoluble residue was sequentially extracted as described for muscle.

Residues in eggs were extracted with acetone and the acetone-soluble fraction was partitioned between hexane and acetonitrile. The acetone-insoluble material was extracted with methanol. The acetonitrile- and methanol-soluble residues were combined and characterized by silica gel column chromatography. The methanol-insoluble material was sequentially extracted with methanol:ammonium hydroxide (9:1), methanol:sodium hydroxide (3:1), and methanol:water:sodium hydroxide (6:1:1).

The distribution of trifluralin residues in tissues and eggs from hens dosed at 1,000x, expressed as a percentage and concentration (ppm) of the total radioactive residues (TRR), is presented in Table 8. [The percentages presented for fractionation of residues were based on the total radioactivity recovered in the fractions.]

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Table 8. Radioactivity (ppm trifluralin equivalents) in fractions from tissues and eggs of hens dosed with [^{14}C]trifluralin at 1,000x; the percentages given in parentheses are based on the sum of the radioactivity in the fractions.

Fraction	Muscle	Liver	Skin/fat	Eggs
Methanol/ acetonitrile	0.0522 (36)	0.8712 (35)	0.3182 (68)	0.2579 (50)
Hexane	0.0029 (2)	0.0498 (2)	0.0140 (3)	0.0155 (3)
Hydrolyzed solids				
methanol/base	0.0105 (7)	0.2738 (11)	0.0187 (4)	0.0877 (17)
methanol/water/ base	0.0334 (23)	0.6969 (28)	0.0655 (14)	0.0928 (18)
methanol/acid	0.0145 (10)	0.1742 (7)	0.0608 (13)	--
Nonextractable	0.0319 (22)	0.3485 (14)	0.0234 (5)	0.0464 (9)

Characterization of residues

The acetonitrile/methanol fractions from muscle, liver, skin/fat, and eggs and the methanol/base and methanol/water/base extracts from liver solids were subjected to silica gel column chromatography, eluted with a gradient of increasing polarity prepared using hexane, toluene, ethyl acetate, methanol, water, and glacial acetic acid. Some of the radioactive fractions eluted from silica gel were analyzed using thin-layer chromatography (TLC) and/or high performance liquid chromatography (HPLC). TLC plates were developed using chloroform:methanol:acetic acid (90:10:2) or hexane:methanol (98:2), and radioactivity was located by autoradiography. Compounds were eluted from HPLC using acetonitrile and/or acetonitrile:water:5% ammonium acetate (30:60:10).

Residues in muscle separated into three radioactive peaks on silica gel. Based on the elution pattern, one compound was tentatively identified as unchanged trifluralin, accounting for ca. 1% of the TRR. No other residues were characterized.

Acetonitrile/methanol-soluble residues from liver resolved into three major peaks and numerous minor fractions. One peak, eluting where TR-2 and TR-4 were expected, contained 2% of the TRR; two other peaks each contained 3%. Attempts to identify the ^{14}C-residues using TLC were unsuccessful. Attempts were made to isolate and identify the ^{14}C-activity in the one of these fractions. The residue remained in water during chloroform

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partitioning but was extracted from water by multiple extractions with ethyl acetate. It was not found by TLC or HPLC to be identical to any of the available standards, though it most closely resembled TR-19.

One radioactive fraction from column chromatography, accounting for 15% of skin/fat residues, eluted where trifluralin was expected, although the identity was not confirmed. A fraction comprising 5% of the TRR reportedly behaved as TR-2 in TLC and HPLC; data to support this contention were not provided.

Fractionation of radioactivity in eggs resulted in peaks expected, based on elution behavior, to contain TR-3, TR-5, TR-6, TR-7, and TR-14.

The identified and putative metabolites of trifluralin in animals and plants are depicted in Table 1. It should be noted that all of the identifications of components in poultry tissues and eggs are considered putative.

Recovery of trifluralin

Samples of liver, skin/fat, muscle and eggs were fortified with [^{14}C]trifluralin (10.8 dpm). Recovery of radioactivity in edible tissues ranged from 84 to 121% when added at 0.005 ppm. In eggs, recovery of radioactivity, added at 0.001 ppm was 78%.

Summary of poultry metabolism

Radioactive residues were <0.003 ppm (nondetectable) each in poultry muscle and skin plus fat, 0.004 ppm in liver, and <0.001 ppm (nondetectable) in eggs from hens administered [^{14}C]trifluralin at a level in the diet equivalent to 1x the maximum theoretical dietary intake of 0.05 ppm. Residues in tissue and egg samples from hens dosed at 1,000x were fractionated and the solids hydrolyzed, but attempts to identify metabolites using TLC or HPLC were unsuccessful. The registrant postulated, based on the polarities of residues separated on silica gel column chromatography, that trifluralin could be present in muscle; TR-2, TR-4, and TR-19 could be present in liver; skin/fat could contain trifluralin and TR-2; and that TR-3, TR-5, TR-6 TR-7, and TR-14 might be present in eggs. More thorough characterization of radioactive residues in poultry tissues and eggs is not required given the low levels expected to result from the maximum theoretical dietary intake. However, the registrant needs to submit additional data regarding the dosing regimen, specifically, the weights of the hens and the amount of feed consumed per day.